Chemical Composition of *Pistacia terebinthus* L. and its Phytochemical and Biological Properties

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Vitamin C, enzyme activities, phenolic compounds, antioxidant capacity, antimicrobial activity, and essential oil analyses of ripe and unripe fruits of *P. terebinthus* were investigated. Vitamin C amounts of ripe and unripe fruits were 63.2 and 15.4 mg/100g, respectively. The main phenolic compounds of unripe and ripe fruits are rutin, syringic acid, and gallic acid. It was determined that the enzyme inhibitor activities in the ripe and unripe fruits were 0.136 mg/mL and 2.14 mg/mL. In all of the free radical scavenging (DPPH and ABTS) activity, ferric (III) ion reducing antioxidant power (FRAP) capacity, total phenolic substance amounts (TPC), total flavonoid substance amounts (TFC), and total antioxidant activity (TAC) antioxidant methods analyzed with plant parts, the methanol extracts obtained from the ripe fruits of the *P. terebinthus* showed higher antioxidant properties than the methanol extracts obtained from the unripe fruits. *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 showed antimicrobial activity against microorganisms, while methanol extracts obtained from unripe fruit samples did not show antimicrobial activity against the microorganisms used. The chemical grade with the most compounds in the essential oils of *P. terebinthus* were monoterpenoids, sesquiterpenes, and monoterpenes in unripe and ripe fruits, respectively. The main components were α-pinene with 22.8% and 27.3% ratios in unripe and ripe fruits, respectively.

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INTRODUCTION

Pistacia terebinthus, belonging to the family Anacardiaceae, is a long-lived plant (Özcan *et al.* 2020; Akpulat *et al.* 2021). It is one of 20 *Pistacia* species extensively distributed in the United States, Asia, and the Mediterranean (Couladis *et al.* 2003; Kavak *et al.* 2010; Bozorgi *et al.* 2013; Özcan *et al.* 2020). It is popularly known as 'menengic' in Türkiye (Bozorgi *et al.* 2013; Uysal *et al.* 2022). Terebinth coffee is one of the most popular herbal coffees in Türkiye. This coffee is commonly known as "menengic coffee" in Türkiye (Orhan *et al.* 2012). The *P. terebinthus* plant is shown in Fig. 1. Medicinal plants, because of their bioactivity and nourishment features, arise as an alternative to synthetic products. They are used not only in conventional medicine but also in several pharmaceutical, ornamental, and food products (Foddai *et al.* 2015; Rauf *et al.* 2017).

Fig. 1. *P. terebinthus* plants (Photo: Mehmet ÖZ, 19.09.2021)

Most of the plant parts with the inclusion of resin, fruit fatty oil, and fruits are used as traditional medicine and food in the area (Baytop 1999; Stanojević *et al.* 2021). Plants are significant resources of potential phytochemicals, for instance, vitamins and polyphenols (Uysal *et al.* 2022). The small fruits of *P. terebinthus* are spherical nuttlets that are dark greenish when ripe. Its fresh fruits and shoots are used for human nutrition (Özcan *et al.* 2009). *P. terebinthus* is consumed as a snack food, used in soaps, or in making a coffee-like drink after roasted and ground (Topcu *et al.* 2007; Kavak *et al.* 2010; Durmaz and Gökmen 2011; Gogus *et al.* 2011; Orhan *et al.* 2012). The fruits are also used in the baking of a coffee substitute and as a specialty village bread (Özcan *et al.* 2009). *P. terebinthus* fruits are important sources of fiber, minerals, oil, and protein. The oil extracted from terebinth fruits is an alternative to herbal oils because it includes high amounts of omega-3 (linoleic acid) fatty acids and mono-unsaturated (oleic acid), and it has desirable taste and odor (Bozorgi *et al.* 2013; Özcan *et al.* 2020).

Moreover, the fruits of *P. terebinthus* are used in traditional medicine for diarrheic, throat infections, gastralgia, antiseptic for bronchitis, stimulant, rheumatism, diuretic, antitussive, wounds, urinary inflammations, burns, cough, stomachache, asthma, and eczema (Topcu *et al.* 2007; Özcan *et al.* 2009; Kavak *et al.* 2010; Durmaz and Gökmen 2011; Gogus *et al.* 2011; Orhan *et al.* 2012; Özcan *et al.* 2020).

Pistacia species have antioxidant activity as well as anti-inflammatory, cytotoxic, and antimicrobial properties due to their high phenolic, tocopherols, and flavonoids contents (Kavak *et al.* 2010; Durmaz ve Gökmen 2011; Durak and Uçak 2015; Özcan *et al.* 2020).

Many studies on the volatile oil constituents of *P. terebinthus* have been made and results have been observed in literature. These studies found α-pinene, β-pinene, sabinene, limonene, *p*-cymen-8-ol, terpinen-4-ol, and caryophyllene as major compositions (Couladis *et al.* 2003; Usai *et al.* 2006; Özcan *et al.* 2009; Orhan *et al.* 2012).

Phenolic compounds, bioactive properties, fatty acids, physicochemical features, essential oils, mineral compounds, antioxidant and antimicrobial features, biological activities, and phytochemicals of terebinth plants have been studied by various researchers (Ozcan *et al.* 2009; Kavak *et al.* 2010; Durmaz and Gökmen 2011; Gogus *et al.* 2011;

Orhan *et al.* 2012; Rauf *et al.* 2017; Mollica *et al.* 2018; Zengin *et al.* 2018; Uysal *et al.* 2022). Nonetheless, there have been no studies that compare the features, antimicrobial properties, phenolic compounds, enzyme activity, and vitamin C profiles of different ripe and unripe fruits of *P. terebinthus*.

In this study, the effects of vitamin C, enzyme activities, phenolic compounds, antioxidant capacity, antimicrobial activity, and essential oil in ripe and unripe fruits of terebinth plants were determined. It is the first study that has been done with the terebinth plants located in the north of Türkiye. In terms of chemistry, it is a combined study in which enzyme inhibition, phenolic and volatile compounds, vitamin C, and antimicrobial-antioxidant analysis are performed.

EXPERIMENTAL

Plant Material

In this study, *P. terebinthus* unripe and ripe fruit (1000 g) samples were gathered in Torul-Köstere Village (40°36'28"N, 39°19'18"E, Altitude: 1045 m) located within the borders of Gümüşhane Province, Türkiye. The locations where the samples were taken are shown in Fig. 1. The taxonomic diagnosis of plant samples was determined by Assoc. Prof. Mutlu Gültepe, in the Department of Forestry, Dereli Vocational School, Giresun University, Giresun, Türkiye. The plant was saved with the number KTUB Gültepe 718 with identification from the Herbarium of Karadeniz Technical University, Faculty of Science, Department of Biology. Fruit samples were collected by hand on 19.09.2021 (autumn season) from the specified region and dried in the shade by mixing at regular intervals. Samples were stored in a cool, dry place out of the sun until analysis. For analysis, the fruits were ground and not treated differently.

Extraction Procedure

Essential oil extraction

The essential oils were obtained by hydrodistillation method in the Clevenger device. Sufficient amounts (100 g) of plant parts from unripe and ripe fruit samples were cut into pieces and placed in skewered balloons and distilled for 4 h by adding 500 mL of distilled water (Küçük *et al.* 2006). The essential oils acquired were dissolved in 1 mL of high performance liquid chromatography (HPLC) grade hexane, dried with anhydrous sodium sulfate, filtered, and stored in an indoor brown bottle at -18 °C until the analysis was completed.

Extraction of methanol

The extraction process was performed using an ultrasonic bath (3 L 320 W Bandelin Ultrasonic Bath). After the fruit parts were ground and 10 g were taken, 50 mL of 80% aq. MeOH was added, and then an ultrasound-assisted extraction process was applied at 60 min and 40 °C. At the end of 60 min, it was filtered 2 times through Whatman 1 filter and centrifuged at 4000 rpm for 10 min and plant extracts were obtained. At the end of centrifugation, the upper part was taken into a beaker, and the extracts were obtained by completely evaporating the methanol at 40 °C (Dranca and Oroian 2016).

Analysis of Vitamin C in Extracts

Vitamin C analyses of the samples were performed using an HPLC-UV device with a UV 1000 detector according to high pressure liquid chromatography UV detector method. The analytical column RP C18 (250 x 4,6 mm, 5 µm) was used with the mobile phase: methanol: water (5:95, v/v) pH= 3 (H_3PO_4), flow 1 mL/min, injection volume 20 µL, with detection of UV at 254 nm. For the calibration curve, standard solutions of 10, 30, 60, 90, and 120 mg/L concentrations were prepared from L-ascorbic acid. A total of 10 g of the ripe and unripe fruits of terebinth plants were taken and divided into pieces in a shredder. Then, 70 mL sufficient amount of metaphosphoric acid (15% m/m) was added to the smashed fruits and mixed in the homogenizer. The homogenized samples were completed to 100 mL and filtered through filter paper. After the filtrates were passed through a 0.45-micron filter, they were taken into vials and given to the HPLC device. The amount of analyzed vitamin C in the sample was calculated using the calibration graph method (y = 9498.7 x - 4236) (Öz *et al.* 2018).

Enzyme Inhibitory Activities of the Extracts

The α-glucosidase inhibitory activity of the samples was studied through modification (Yu *et al.* 2012). In the study, first, 650 µL of phosphate bumper (pH: 6.8 and 0.1 M) was added to the test tubes. Then, 20 µL of sample and 30 µL of α-glucosidase enzyme (*Saccharomyces cerevisiae*, lyophilized powder ≥ 10 units/mg protein) prepared in phosphate buffer were added. After the mixture was incubated at 37 °C for 10 min, 75 µL of substrate (4-nitrophenyl-*α*-D-glucopyranoside) was added. After the mixture was kept at 37 °C for 20 min, 650 µL of 1 M Na_2CO_3 was added to all tubes and the reaction was stopped. The absorbance value was read at 405 nm in an ultraviolet/visible (UV/VIS) spectrophotometer. Different densities of acarbose (positive control) were studied as the standard inhibitor. The study was performed in three parallel and reagent-sample blanks. The IC_{50} values of acarbose and samples (sample concentration that halves the enzyme activity present in the environment) were calculated.

Determination of Phenolic Compounds

All samples were ultrasonically bathed for 20 min and filtered through a syringe filter (0.45 µm) before analysis. Chromatographic analysis of dry methanol extracts of unripe and ripe samples was used Agilent 1260 Infinity HPLC-DAD system (Agilent Technologies, Waldbronn, Germany) device. The gallic acid, 4-hydroxybenzoic acid, caffeic acid, sesamol, paracoumaric acid, benzoic acid, protocatechuic acid, catechin, syringic acid, vanillin, syringaldehyde, rutin, protocatechuic aldehyde, vanillic acid, rutin, ferulic acid, coumarin, epicatechin, rosmarinic acid, t-cinnamic acid, quercetin, kaempferol, and chyricin were used as standards for the analysis. The analysis method of the phenolic compounds of the samples was studied by modifying the gradient flow of the mobile phase with some changes (Paje *et al.* 2022). Chromatographic separation of individual components was performed using a Hypersil HPLC Column (250 x 4.6 mm^2, 5µm). Mobile phase solvent A was used as mixture 0.5% acetic acid in water (0.5: 95.5, v/v) and ACN (solvent B). The gradient elution was started with 95% of solvent A and reduced to 75% after 20 min. Solvent A was reduced to 50% at 45 min and to 10% at 55 min. It was then increased to 65% at 65 min and continued for up to 70 min. The injection volume was 10 µL, and the flow rate was 1.0 mL/min. The wavelengths used in the DAD detector were 240, 250, 254, 280, and 324 nm.

Determination of Antioxidant Capacity

The antioxidant capacities of the attained methanol extract were detected according to free radical scavenging (2,2-diphenyl-1-picrylhydrazil (DPPH) and ABTS) activity and ferric (III) ion reducing antioxidant power (FRAP) capacity methods. Additionally, some bioactive component amounts were shown by total phenolic substance amounts (TPC), total flavonoid substance amounts (TFC), and total antioxidant activity (TAC) works.

Free radical scavenging activity (DPPH)

The DPPH of unripe and ripe fruit methanol extract was obtained using DPPH according to the Sanchez-Moreno method (Sağdıç *et al.* 2011). The method was applied by mixing the methanol extract and DPPH solutions with specific concentrations by vortexing and keeping them at room heat and in the dark for 30 min. In the end of the period, the absorbance of the samples at 517 nm was read and the amount of DPPH remaining in the reaction medium was calculated according to the formula below. Results are given as mg AA eq./g, mg Trolox eq./g, and % free radical removal. The % inhibition was calculated using Eq. 1,

%Inh.=(absorbance of control – absorbance of sample / absorbance of control) x 100 (1)

where Inh. means inhibition. Three measurements for each combination were taken, and the mean values were reported.

Radical cation removal activity (ABTS)

The ABTS radical cation scavenging activity analysis was made using ABTS solution according to the method (Ahmed *et al.* 2015). First, 150 µL of methanol was utilized as the blank. Then, 150 µL of standards (ascorbic acid) were taken and the same procedures were performed. The obtained solution was then read at the spectrophotometer absorbance at 734 nm. The ABTS cation removal activity amounts in the samples were calculated following Ahmed *et al.* (2015). Results are given as mg AA eq./g, mg Trolox eq./g, and % free radical removal. The % inhibition was calculated using Eq. 2,

%Inh.=(absorbance of control – absorbance of sample / absorbance of control) x 100 (1)

where Inh. is Inhibition. Three measurements for each combination were taken, and the mean values were reported.

Ferric (III) ion reducing antioxidant potency (FRAP)

Analysis of the FRAP of methanol extract was made according to the method of Ahmed *et al.* (2015) using FRAP solution. First, 500 µL of distilled water was utilized as blank. Then, 250 µL of the standards were taken and the same procedures were performed. The FRAP amounts in samples were determined as mg $FeSO_4$ equivalent/g using the correct equation of the calibration graph obtained with the $FeSO_4$ solution (Ahmed *et al.* 2015).

Total phenolic substance amount (TPC)

Analysis of the total phenolic content of methanol extract was realized regarding the Kasangana method using Folin-Ciocalteu reagent (Kasangana *et al.* 2015). Then, the prepared mixture was vortexed. Then it was incubated in the dark at room temperature for 120 min. At the end of the incubation period, the absorbance of the mixture at 760 nm was read. A total of 3.7 mL water, 500 µL methanol + 100 µL Folin–Ciocalteu reagent + 600

µL 10% Na_2CO_3 mixture was used as blank. The amounts of phenolic substances in the samples were expressed as mg GA eq./g using the correct equation of the calibration graph obtained with the gallic acid solution.

Total flavonoid substance amount (TFC)

The total flavonoid content of unripe and ripe fruit methanol extract was determined according to the Kasangana method. The absorbance of the resulting mixture was measured in the spectrophotometer at 506 nm. First, 500 µL of distilled water was utilized as blank. Then, 500 µL of the standards were taken and the same procedures were performed. The total amount of flavonoid substances in the samples was made as mg QE eq./g using the equation of the calibration graph obtained with an ethyl alcohol solution of Catechin or Quercetin standards (Kasangana *et al.* 2015).

Total antioxidant amount (TAC)

Analysis of the total antioxidant content of ripe and ripe fruit methanol extract was performed using molybdate reagent according to the Kasangana method. A total of 250 µL of distilled water was used instead of the sample as a blank. The absorbance of the resulting reaction mixtures was read in a 695 nm spectrophotometer. Then, 500 µL of the standards were taken and the same procedures were performed. The total amount of antioxidant substances in samples was given as mg GA eq/g using the correct equation of the calibration graph obtained with the solution of ascorbic acid (Kasangana *et al.* 2015).

Determination of Antimicrobial Activity

Microorganisms utilized in the work were obtained from the laboratory of Gümüşhane University, Department of Food Engineering. The antimicrobial activities of the essential oil and methanol extracts were detected by disk diffusion method against 13 microorganisms, including 10 bacteria and 3 yeast-molds (Matuschek *et al.* 2014). Antimicrobial activity was realized in two phases: preparation of bacteria and yeasts and preparation of examples. Bacteria were used in a Nutrient Broth medium after 24 h of the first activation at 36 °C and after 18 h of the second activation at 36 °C. Then, 1% of the microorganisms to be used in the study were added to the prepared sterile solid media and they were poured into petri dishes and allowed to solidify. Then, 5-mm diameter wells were opened on the solidified media. The incubation process was carried out by adding the solutions of the essential oil prepared with hexane to the opened wells. Petri dishes containing bacteria were incubated for 24 h at 36 °C, and petri dishes containing mold and yeast were incubated for 48 h at 27 °C. In the end of the determined period, the results were found by measuring the transparent zones around the discs.

GC-MS/FID Conditions for Essential Oil Analysis

The essential oil obtained by the hydrodistillation method in the Clevenger system was dissolved in hexane, passed through a 0.45-micron filter, placed in amber colored vials, and placed in the autosampler. After the volatile compounds were separated on the gas chromatography column, the mass spectra of each were individually taken in the mass spectrophotometer, and their structures were elucidated by comparing the mass spectra of each component with the reference components of the Willey and NIST libraries. To confirm the detected compounds, the Kovats indices of the compounds were compared with the literature data (Adams 2007). The measurement of the essential oil was made with the gas chromatography flame ionization detector (Agilent Technologies Inc, Santa Clara,

CA, USA). For GC, the split ratio was adjusted as 1:5 by injecting 1 µL of essential oil in hexane into the same column. The GC-MS/FID analyses were performed on Agilent-7890 model device and an HP-5 model apolar capillary column (30 m x 0.32 mm, film thickness 0.25 µm) was used for analysis. The injector, ion source and quadrupole rod temperatures were 250 °C, 230 °C, and 150 °C, respectively. Injections were applied in split (25:1) mode using helium (>99.999%) as the carrier gas with a flow rate of 1 mL/min. Then, 1 µL of essential oil solution in hexane (GC class) was injected and initially the GC oven temperature program kept at 60 °C for 2 min, increased to 240 °C with a rise of 3 °C/min, and spectra were obtained. Mass spectra were acquired at a scan speed of 2 spectra per second after a solvent delay of 3.8 min, and the mass scan range was set at m/z 45–450. The FID detector temperature was maintained at 250 °C with a hydrogen flow of 35 mL/min and air flow of 350 mL/min.

RESULTS AND DISCUSSION

The Analysis of Vitamin C in Extracts

The amount of vitamin C in the unripe fruit was 63.15 ± 0.45 mg/100 g, and the amount of vitamin C in the ripe fruit was 15.44 ± 0.13 mg/100 g. The amount of vitamin C in unripe fruits was higher than the amount of vitamin C obtained from ripe fruits. In terms of vitamin C, there is no study in the literature on the *P. terebinthus* plant. In the vitamin C analysis performed on unripe and ripe fruits of *Pistacia lentiscus* belonging to the same family, Ayad *et al.* (2023) reported 1.23±0.03 in red berries and 1.25±0.01g AA/100 g in black berries.

The amount of vitamin C detected in fruits decreased in ripe fruits. The content of vitamin C in vegetables and fruits can be influenced by various factors such as preharvest climatic conditions and cultural practices, maturity and harvesting methods, and postharvest handling, genotypic differences procedures (Medveckienė *et al.* 2021). Medveckienė *et al.* (2021) stated that the amount of vitamin C in rosehip fruits tended to decrease with the ripening stage.

Enzyme Inhibitory Activities of the Extracts

Enzyme inhibitor activity was 0.136 mg/mL in unripe fruits of *P. terebinthus*, and enzyme inhibitor activity was 2.14 mg/mL in ripe fruits. It has been determined that unripe fruits are more effective in enzyme inhibition.

Even though the terebinth plant was considered a significant plant, there was no study on the extensive enzyme inhibitory activity. In terms of the foregoing expressions, the authors determined enzyme inhibitory activity.

Yu *et al.* (2012) stated that a lower IC_{50} value of the sample results in more effective enzyme inhibition. The methanol extract also analyzed remarkable enzyme inhibitory activity. The outcomes suggest that the extracts in leaves of *P. terebinthus* have for use as a good nominee of native enzyme inhibitors and antioxidants (Uysal *et al.* 2022). In α-glucosidase inhibition assay, water (14.5 mmol ACAE/g) and methanol (14.6 mmol ACAE/g) extracts showed parallel activity. The enzyme inhibition outcomes of samples may be noticeable by flavonoid and phenolics constituents. Similarly, they determined the maximum BChE activity in the methanol extract of *P. terebinthus* leaves (Uysal *et al.* 2022). In recent years, many studies demonstrated phenolic constituents in plants play a significant role in enzyme inhibition (Khan *et al.* 2018; Kim *et al.* 2020; Yang *et al.* 2021).

Some reports demonstrated distinct parts of terebinth fruits that indicated enzyme inhibitory activity. For instance, methanol fruit extract indicated the finest BChE inhibitory effect (45.74% at 200 μg/mL) (Orhan *et al.* 2012). Additionally, fruits of *P. terebinthus* indicated anticholinesterase activity (Hacıbekiroğlu *et al.* 2015). The oil of terebinth plants displayed antidiabetic activities in diabetic rats (Uyar and Abdulrahman 2020). Enzyme inhibitory activities in the extracts of unripe and ripe terebinth fruits are demonstrated in Table 1.

Table 1. Amount of Enzyme Inhibitory Activities in Fruit Samples of *P. terebinthus*

Enzyme Inhibitory Activities		
	IC_{50} (mg/mL)	R^2
Akarboz	0.021 ± 0.02	0.9911
Unripe fruit	0.136 ± 0.09	0.9967
Ripe fruit	2.138 ± 0.17	0.9953

Table 2. Phenolic Compounds in Fruit Samples of *P. terebinthus*

Number	Compounds	Unripe Fruit (mg/kg)	Ripe Fruit (mg/kg)
1	Gallic Acid	197.86	2052.72
2	4-Hydroxybenzoic Acid	<LoQ	<LoQ
3	Caffeic Acid	<LoQ	<LoQ
4	Sesamol	<LoQ	<LoQ
5	Paracoumaric Acid	<LoQ	<LoQ
6	Benzoic Acid	<LoQ	<LoQ
7	Protocatechuic Acid	<LoQ	<LoQ
8	Catechin	<LoQ	<LoQ
9	Syringic Acid	465.71	1669.00
10	Vanillin	<LoQ	<LoQ
11	Syringaldehyde	<LoQ	<LoQ
12	Rutin	4527.06	10705.58
13	Protocatechuic Aldehyde	<LoQ	<LoQ
14	Vanillic Acid	<LoQ	<LoQ
15	Ferulic Acid	<LoQ	<LoQ
16	Coumarin	<LoQ	<LoQ
17	Epicatechin	<LoQ	<LoQ
18	Rosmarinic Acid	<LoQ	<LoQ
19	*t*-cinnamic Acid	<LoQ	<LoQ
20	Quercetin	<LoQ	<LoQ
21	Kaempferol	<LoQ	<LoQ
22	Chyrisin	<LoQ	<LoQ

*LoQ (limit of quantitation) value: 0.1 mg/kg

Determination of Phenolic Compounds

The major phenolic components of unripe fruit samples were rutin (4530 mg/kg), syringic acid (466 mg/kg), and gallic acid (4200 mg/kg). The main phenolic compounds of ripe fruit samples were rutin (10700 mg/kg), gallic acid (2050 mg/kg), and syringic acid

(1670 mg/kg). The main phenolic compounds of unripe and ripe fruits are rutin, syringic acid, and gallic acid. Phenolic components of unripe and ripe terebinth fruits are shown in Table 2. In the present study, the amounts of rutin, syringic acid, and gallic acid were found to be high in ripe fruits. It can be thought that the antimicrobial properties of the ripe fruit may be due to these substances. Dubey *et al.* (2013) declared that rutin is a good antibacterial and antifungal agent as a result of their studies. Gallic acid may play a protective role in healthy individuals by inhibiting apoptosis and a naturally occurring gallic acid is highly antioxidant (Zahrani *et al.* 2020). Lima *et al.* (2016) reported that gallic acid and its methyl ester showed antibacterial activity on *Bacillus subtilis, Staphylococcus aureus,* and *Escherichia coli*.

Determination of Antioxidant Capacity

When the antioxidant capacity amounts are examined, the DPPH amount was 73.1 mg AA eq./g in *P. terebinthus* unripe fruit methanol extract and 133.2 mg AA eq./g in ripe fruit methanol extract. The DDPH % inhibition rates of the same samples were between 89.7% and 71.7%. When the DPPH amount and DPPH % inhibition rates of unripe and ripe fruit methanol extracts are compared, it can be said that the DPPH amount of ripe fruit methanol extract was higher than that of unripe fruit methanol extract and the DPPH % inhibition rates of unripe fruit methanol extract was higher than that of ripe fruit methanol extract.

When the DPPH amount and DPPH % inhibition rates of unripe and ripe fruit methanol extracts are compared, it can be said that methanol extract of ripe fruit was higher DPPH amount and methanol extract of unripe fruit was higher DPPH % inhibition rate.

In the authors' study, ABTS amounts of the samples were 81.5 mg AA eq./mg in unripe fruit methanol extract and 134.26 mg AA eq./mg in ripe fruit methanol extract. ABTS % inhibition rates were 97.2% in unripe fruit methanol extract and 71.1% in ripe fruit methanol extract. The ABTS capacity of ripe fruit methanol extract was higher than that of unripe fruit methanol extract. The amount of FRAP in unripe and ripe fruit methanol extract was 18.4 mg $FeSO_4$ eq./g and 18.7 mg $FeSO_4$ eq./g, respectively (Table 3).

The authors proposed that some flavonoid derivatives can account for the high antioxidant capacity of the fruit extracts (Orhan *et al.* 2012). In a work on acetone and methanol extracts acquired from the fruits of terebinth plants, the extracts were analyzed for their antioxidant capacity and both extracts demonstrated a high DPPH and superoxide radical scavenging activity (Topçu *et al.* 2007). The antioxidant content outcomes demonstrated that methanol extract (DPPH: 2.06 mmol TE/g, ABTS: 3.29 mmol TE/g, FRAP: 1.62 mmol TE/g) exhibited promising antioxidant capacity (Uysal *et al.* 2022). DPPH of the extracts varied over a wide range (8.86% to 64.43%) and were correlated with TPCs (Durak and Uçak 2015). This study is in compliance with the authors' data as the extracts attained in the work have a notable scavenging effect against DPPH radical. The outcomes suggest that the extracts of terebinth plant leaves have the potential to be utilized as a good nominee of natural antioxidants.

Examining the bioactive compounds

In this study, while the total phenolic content of unripe fruit methanol extract was 41.5 mg GA eq./g, it was 47.3 mg GA eq./g in ripe fruit methanol extract. *P. terebinthus* total phenolic content was higher in ripe fruit methanol extract than in unripe fruit samples.

In this work, the total flavonoid substance amount of unripe fruit methanol extract was 88.3 mg QE eq./g, while it was 169 mg QE eq./g in ripe fruit methanol extract. The total flavonoid content of the samples was high. In the authors' research, the total antioxidant content of unripe and ripe fruit methanol extract in the samples was 45.9 mg GA eq./g and 72.8 mg GA eq./g, respectively. As shown in Table 3, the amount of total antioxidant substance was higher in ripe fruit methanol extract.

It is common for ultrasonic extraction to enhance the quantities of phenolic constituents in terebinth fruit extracts. Total phenolic quantities of examples ranged from 84.0 to 87.3%. Among the sonicated samples, the highest total phenolic (251 mg/100 g) amounts were obtained in examples sonicated for 30 min (Özcan *et al.* 2020). The increased amounts of phenolic constituents in sonicated *P. terebinthus* samples over the control could be due to the deterioration of the cell wall by sonication breakdown of the material, that leads to the release of more phenolic constituents (Abid *et al.* 2013). Additionally, Ma *et al.* (2009) demonstrated that ultrasonic treatments greatly enhanced the extraction of various phenolic constituents from citrus peels.

Total flavonoid and phenolic amounts in methanol extracts of the terebinth plants were 123 µg PEs/mg extract and 22.6 µg QEs/mg extract, respectively (Topçu *et al.* 2007). Total flavonoid and phenolic amounts of *P. terebinthus* fruit were 47.0 mg quercetin/g and 241 mg GAE/g, respectively (Orhan *et al.* 2012). Total flavonoids and phenolics amounts of the extracts were 65.5 to 211 mg GAE/g and 54.9 to 170 mg RE/g, respectively. The maximum level of TPC was detected in the methanol extract. The methanol extract (211 mg GAE/g) had the highest content TPC compared with other extracts (Uysal *et al.* 2022). The difference between this article and the literature studies may be because of the distinction in the phenolic profiles of the fruits. The previous and current studies clearly demonstrate that TPCs of *P. terebinthus* extracts differ greatly from each other. These differences can be due to factors such as fruit species and gathering sites (Farhat *et al.* 2013). Environmental and climate circumstances are also substantial factors affecting the TPCs of examples. Furthermore, a higher phenolic amount was demonstrated in unripe fruits than in ripe ones (Costa *et al.* 2013).

When the antioxidant analyses of unripe and ripe fruit methanol extracts were examined, it was observed that DPPH, FRAP, TPC, TFC, and TAC antioxidant values of fruit methanol extract were higher in ripe fruit methanol extract, except for ABTS. In this study, when the antioxidant capacities of the authors' examples are estimated, it is seen that they are compatible with the literature. This situation shows parallelism with antimicrobial activities.

The extract attained from the *P. terebinthus* leaf has noticeably higher antioxidant activity when compared with the other natural and synthetic antioxidants. This may conceivably be because of the high phenolic and flavonoid compounds content of crude extract (Kavak *et al.* 2010). Between the extracts, methanol extract had the most powerful antioxidant capacity. These observations suggest that the phytochemical amounts in the methanol extract can be accountable for the major part of antioxidant capacity, as described in former studies (Kavak *et al.* 2010; Uysal *et al.* 2022). Former studies indicated that the antioxidant activity of terebinth plant fruits may be affected by main flavonoids and phenolics for example quercetin (Topçu *et al.* 2007; Uysal *et al.* 2022). Antioxidant analysis results of methanol extracts of ripe and unripe fruits of *P. terebinthus* plant are given in Table 3.

Table 3. Antioxidant Activity Contents and Bioactive Compounds of Essential Oil Attained from Fruit Samples of *P. terebinthus*

		DPPH		ABTS		FRAP mg $FeSO_4$ eq./g	
		Unripe fruits	Ripe fruits	Unripe fruits	Ripe fruits	Unripe fruits	Ripe fruits
Antioxidant Capacity Amounts	**mg AA eq./g**	73.12* ± .39**	133.24 ± 10.05	81.50 ± 3.20	134.26 ± 11.64	18.40 ± 0.05	18.74 ± 0.70
	mg Trolox eq./g	88.24 ± 0.47	161.23 ± 12.00	118.98 ± 4.50	198.74 ± 16.38		
	% Inhibition	89.66 ± 0.47	71.68 ± 5.33	97.25 ± 0.34	71.13 ± 5.41		
		TPC mg GA eq./g		**TFC mg QE eq./g**		**TAC mg GA eq./g**	
Bioactive Components		Unripe fruits	Ripe fruits	Unripe fruits	Ripe fruits	Unripe fruits	Ripe fruits
		41.50 ± 2.01	47.31 ± 3.48	88.31 ± 6.42	169.01 ± 31.47	45.89 ± 0.82	72.75 ± 1.14

*: Means, ** ± : Standard deviation

Determination of Antimicrobial Activity

The results demonstrate that *P. terebinthus* ripe fruit methanol extracts showed antimicrobial activity. However, the essential oil samples of the unripe and ripe fruits of the terebinth plant did not show any antimicrobial activity against the microorganisms used.

It was determined that the ripe fruit of *P. terebinthus* formed zones of 4.24 mm diameters against *Escherichia coli* ATCC 25922 and 4.20 mm diameters against *Staphylococcus aureus* ATCC 25923. The methanol extract of the unripe fruits of the terebinth plant did not show any antimicrobial activity against the microorganisms used. Chemical variability may be due to genetic or environmental factors dependent on geographical and edaphoclimatic conditions, leading to the occurrence of different chemotypes as infraspecific chemical races (Llorens-Molina and Vacas 2015).

This study demonstrates the antimicrobial effects of methanol extract samples in ripe fruits of terebinth plant against *Escherichia coli* and *Staphylococcus aureus*. Of these, methicillin-resistant *Staphylococcus aureus* is known to be the cause of hospital-acquired infections and the main factor in community-acquired infections. This feature has been understood as a result of the emergence and increase in the frequency of infections acquired from the community.

Hospital infections are difficult to treat, expensive, and can involve drug-resistant microorganisms (Öztürk 2008). Therefore, it is not easy to treat a nosocomial infection, and antibiotics that counteract this infection have not been strong in the face of a nosocomial infection. The extract of the *Pistacia terebinthus* plant inhibited *Staphylococcus aureus* bacteria causing this infection with a zone diameter of 14 mm. For this reason, Akpulat *et al.* stated that *Pistacia terebinthus* plant can be used in the treatment of hospital infection by conducting necessary studies on its galls (Akpulat *et al.* 2021). The results of the essential oil samples showing antimicrobial activity are given in Table 4.

Table 4. Antimicrobial Activity of Crude Extract of *P. terebinthus* Ripe Fruits

Ripe Fruits*				
Bacteria Species	250 ppm	500 ppm	1000 ppm	5000 ppm
Bacillus subtilis ATCC 6633	-	-	-	-
Enterococcus faecalis ATCC 29212	-	-	-	-
Bacillus cereus ATCC 9634	-	-	-	-
Aeromonas hydrophila ATCC 35654	-	-	-	-
Escherichia coli O157:H7 35150	-	-	-	-
Listeria monocytogenes ATCC 7644	-	-	-	-
Staphylococcus aureus ATCC 25923	-	-	4.20 ± 0.01	4.15 ± 0.01
Shigella flexneri ATCC 12022	-	-	-	-
Escherichia coli ATCC 25922	-	-	-	4.24 ± 0.01
Salmonella typhimurium ATCC 23566	-	-	-	-
Yeast-Mold				
Saccharomyces cerevisiae S288C	-	-	-	-
Aspergillus flavus ATCC 46283	-	-	-	-
Candida albicans ATCC 10231	-	-	-	-

*expressed as inhibition zone in mm

GC-MS/FID Conditions for Essential Oil Analysis

After analysis of essential oils by GC-MS/FID methods, the structure of a total of 98 components was detected in the unripe fruits of *P. terebinthus*, but the structure of 4 components could not be identified. The highest percentages of essential oils isolated from unripe fruits were *α*-pinene (22.81%), *α*-terpinolene (20.79%), and cis-*β*-ocimene (9.41%). The most abundant parent compound in unripe fruit samples is α-pinene. In the essential oil of the ripe fruits of terebinth plants, the structure of a total of 71 compounds was elucidated. The highest *α*-pinene (27.27%), *α*-terpinolene (17.11%), and trans-*β*-ocimene (10.73%) were the main compounds in ripe fruit samples. It is understood that the most common main compound in ripe fruit samples is *α*-pinene. It is seen that the main constituents of the essential oil in the ripe and unripe fruits are the same. Because of its antioxidant properties, *α*-pinene and *β*-pinene are widely used in the pharmaceutical industry, as well as in the food, perfumery and cosmetic industries (Kılıç Pekgözlü and Ceylan 2021). Puvača *et al.* (2019) reported that bioactive compounds such as α-terpinene, α-terpinolene and γ-terpinene showed high antioxidant activity. Terpinolene is also used as a synthetic flavoring additive and scent enhancer (Okumura *et al.* 2012).

α-pinene (51.3%), caryophyllene oxide (51.0%), *p*-cymen-8-ol (40.0%), and limonene (39.0%) were the main constituents for distinct places in Türkiye. The main compounds in the examples were caryophyllene oxide, *α*-pinene, p-cymen-8-ol, limonene, sabinene. Twenty-eight constituents representing 92.3 to 100.0% of the oils were defined (Özcan *et al.* 2009). *α*-pinene (26.31%) was dominant in the volatile oil, *cis* (9.34 ± 0.03%) and trans (15.88 ± 0.13%) isomers of *β*-ocimene as well as *D,L*-limonene (14.06 ± 0.21%) were other main compounds in the volatile oil (Orhan *et al.* 2012). Young ripe and unripe fruit oils resulted in the determination of 48 and 51 constituents, respectively. Limonene, *β*-pinene, *α*-phellandrene, and α-pinene were identified as the major compounds of ripe fruit oil. *α*-Pinene, *β*-pinene, limonene, α-phellandrene, and terpinolene were the important

constituents of unripe fruit oil. *β*-Pinene and limonene were determined as the most abundant major components of ripe and unripe fruit oils (Couladis *et al.* 2003). When the results are examined, it can be emphasized that the plant analyzed in this study is a new chemotype of *P. terebinthus* due to the main component differences.

Previous studies realized the fruit volatile oil of *P. terebinthus* underlined the existence of some variations between the authors' findings and those studies. For example, limonene was the main constituent in unripe (34.2%) and ripe (32.8%) fruits of *P. terebinthus* of Türkiye origin (Couladis *et al.* 2003), whereas *α*-pinene was the major constituent in unripe (22.8%) and ripe (27.3%) in the current study. In another study, the fruits of *P. terebinthus* included *α*-pinene (26.31%) as the major constituent, which is more similar to the authors' results (Orhan *et al.* 2012). The fruitful twigs of terebinth plants collected from Sardinia included *α*-pinene (54.8%) as the major constituent, which is more analogous to the current study's outcomes (Usai *et al.* 2006).

Certainly, remarkable variations in the oil constituents might be possibly resulting from climate and locality differences even within the same country, which was also supported by a work studying the effect of locality on constituents, the method of distillation, and oil yields of *P. terebinthus* (Özcan *et al.* 2009). Some variations may be due to the different handling and climatological factors. The volatile oil constituent also varies quantitatively and/or qualitatively with ripening and collection times (Couladis *et al.* 2003). GC/MS-GC/FID analysis outcomes of essential oils attained from ripe and unripe fruits of *P. terebinthus* are shown in Table 5.

Table 5. Percentage Component of Essential Oils from Ripe and Unripe Fruits of *P. terebinthus*

No	RT (min)	Compound Classification	Compounds	% Concentration		RI	LRI
				Unripe	Ripe		
1	5.01	Hydrocarbon	Heptane	0.19	0.07	700	700
2	5.25	Other	2,5-Dimethyltetrahydro furan		0.02	710	727
3	5.49	Hydrocarbon	Methyl cyclohexane		0.13	720	720
4	5.89	Other	1-Methyl-1H-pyrrole	0.02	0.35	737	737
5	6.53	Hydrocarbon	Toluene	0.01	0.01	764	764
6	7.41	Aldehyde	Hexanal		0.10	800	800
7	7.42	Hydrocarbon	Octane	0.34		801	800
8	9.31	Aldehyde	(*E*)-2-Hexenal	0.01	0.01	851	851
9	11.22	Aldehyde	Heptanal	0.02	0.02	901	901
10	12.14	Monoterpene	Tricyclene	0.16	0.17	921	921
11	12.39	Monoterpene	*α*-Thujene	0.12	0.09	927	927
12	12.83	Monoterpene	**α-Pinene**	**22.81**	**27.27**	936	936
13	13.36	Monoterpene	Camphene	0.96	0.89	948	948
14	13.60	Monoterpene	2,4(10)-Thujadiene	0.04	0.01	953	953
15	13.70	Aldehyde	(*E*)-2-Heptenal	0.08		955	955
16	14.55	Monoterpene	Sabinene	0.73	2.37	974	974
17	14.71	Monoterpene	*β*-Pinene	4.40	5.02	977	977
18	15.37	Monoterpene	*β*-Myrcene	2.46	2.17	991	991
19	16.01	Monoterpene	*α*-Phellandrene	3.14	3.18	1005	1005

20	16.29	Monoterpene	δ 3-carene	2.15	2.14	1011	1011
21	16.58	Monoterpene	*α*-Terpinene	1.69	1.27	1017	1017
22	16.99	Monoterpene	*p*-Cymene	1.08	0.61	1025	1025
23	17.26	Monoterpene	**Limonene**	**7.52**	**7.25**	1031	1031
24	17.72	Monoterpene	***trans*-*β*-Ocimene**	**6.05**	**10.73**	1040	1040
25	17.93	Aldehyde	Benzeneacetaldehyde	0.02		1044	1044
26	18.26	Monoterpene	***cis*-*β*-Ocimene**	**9.41**	**9.44**	1052	1052
27	18.67	Monoterpene	*γ*-Terpinene	0.72	0.20	1060	1060
28	20.30	Monoterpene	**α-Terpinolene**	**20.79**	**17.11**	1094	1094
29	20.50	Monoterpenoid	*α*-Naginatene	0.03	0.04	1097	1095
30	20.64	Monoterpenoid	Linalool	0.18	0.04	1100	1100
31	20.85	Aldehyde	Nonanal	0.14	0.11	1104	1104
32	21.24	Monoterpene	*p*-Mentha-1,5,8-triene	0.07	0.02	1112	1112
33	21.37	Monoterpenoid	Fenchol	0.02		1115	1115
34	21.43	Monoterpene	(*E*)-4,8-Dimethyl-1,3,7-nonatriene	0.18	0.12	1117	1118
35	21.70	Monoterpenoid	*trans*-Sabinene hydrate	0.20	0.05	1122	1116
36	21.94	Monoterpenoid	*α*-Campholenal	0.04	0.01	1127	1127
37	22.12	Monoterpene	*Allo*-Ocimene	2.43	4.34	1131	1131
38	22.40	Aldehyde	Benzenepropanal		0.02	1136	1133
39	22.59	Monoterpenoid	*cis*-Sabinol		0.10	1140	1143
40	22.61	Monoterpenoid	*trans*-Pinocarveol	0.37		1141	1141
41	22.87	Monoterpenoid	(-)-Camphor	0.11	0.08	1146	1145
42	23.06		*Unidentified*	0.13		1150	
43	23.46	Aldehyde	(*E*)-2-Nonenal	0.02	0.01	1158	1158
44	23.72	Monoterpenoid	Pinocarvone	0.07	0.01	1164	1164
45	23.92	Monoterpenoid	Borneol	0.16	0.03	1168	1168
46	24.46	Monoterpenoid	Terpinen-4-ol	0.98	0.09	1179	1179
47	24.83	Monoterpenoid	*p*-Cymen-8-ol	0.27	0.02	1187	1187
48	25.11	Monoterpenoid	*α*-Terpineol	0.59	0.01	1193	1193
49	25.35	Monoterpenoid	Myrtenol	0.22	0.05	1198	1198
50	25.66	Other	2,3,6-trimethyl-phenol		0.01	1204	1203
51	25.68	Other	*o*-Cumenol	0.04		1205	1203
52	25.87	Monoterpenoid	*trans*-Piperitol	0.06		1209	1209
53	26.00	Monoterpenoid	Verbenone	0.02		1212	1212
54	26.11	Aldehyde	(*E,E*)-2,4-Nonadienal	0.05		1214	1214
55	26.42	Monoterpenoid	*cis*-Carveol	0.04		1221	1221
56	26.57		*Unidentified*	0.06		1224	
57	27.57	Monoterpenoid	*D*-Carvone	0.02		1246	1246
58	27.74	Monoterpenoid	*p*-Menth-1(7)-en-2-one	0.02		1249	1238
59	28.10	Monoterpenoid	Piperitone	1.08	0.12	1257	1257
60	28.33	Aldehyde	(*E*)-2-Decenal	0.24		1262	1263
61	29.01	Monoterpenoid	Phellandral	0.04		1277	1276

62	29.50	Monoterpenoid	Bornyl acetate	0.28	0.15	1287	1285
63	29.79	Aldehyde	(*E,Z*)-2,4-Decadienal	0.02		1294	1294
64	30.21	Monoterpenoid	Thymol	0.02		1303	1303
65	30.82	Aldehyde	(*E,E*)-2,4-Decadienal	0.06		1317	1317
66	31.81	Sesquiterpene	Bicycloelemene		0.13	1339	1338
67	31.82	Monoterpene	1,5,5-Trimethyl-6-methylene-cyclohexene	0.11		1340	1338
68	32.33	Sesquiterpene	*δ*-Elemene	0.27	0.18	1351	1351
69	32.89	Aldehyde	2-Undecenal	0.04		1364	1363
70	33.34	Sesquiterpene	*α*-Copaene	0.04	0.13	1374	1374
71	33.53	Sesquiterpene	Ylangene		0.05	1379	1378
72	33.54	Sesquiterpene	*α*-Cubebene	0.08		1379	1379
73	33.97	Sesquiterpene	*β*-Bourbonene	0.18	0.12	1388	1388
74	34.15	Sesquiterpene	*β*-Cubebene	0.01	0.04	1393	1393
75	35.48	Sesquiterpene	Caryophyllene	1.33	0.97	1425	1425
76	36.28	Sesquiterpene	Aromadendrene	0.14	0.04	1444	1444
77	36.48	Sesquiterpene	*γ*-Muurolene	0.04	0.03	1449	1449
78	36.75	Sesquiterpene	*cis*-Muurola-4(15),5-diene	0.09		1455	1455
79	36.91	Sesquiterpene	Humulene	0.62	0.39	1459	1459
80	37.04	Sesquiterpene	Alloaromadendrene	0.02	0.02	1462	1462
81	37.67	Sesquiterpene	*β*-Gurjenene		0.01	1477	1478
82	37.83	Sesquiterpene	*α*-Amorphene	0.15	0.01	1481	1481
83	38.06	Sesquiterpene	Germacrene D	0.83	1.04	1487	1487
84	38.50	Sesquiterpene	Bicyclosesquiphellandrene	0.13	0.02	1497	1498
85	38.67	Sesquiterpene	Bicyclogermacrene	0.37	0.29	1501	1501
86	38.76	Sesquiterpene	*α*-Muurolene	0.08		1504	1504
87	39.05	Sesquiterpene	*δ*-Cadinene	0.06	0.06	1511	1511
88	39.35	Sesquiterpene	*γ*-Cadinene	0.09	0.08	1519	1519
89	39.72	Sesquiterpene	*β*-Cadinene	0.54	0.15	1528	1529
90	39.82	Sesquiterpene	Epizonarene	0.04		1530	1530
91	40.09	Sesquiterpene	Cubenene	0.10	0.03	1537	1537
92	40.52	Sesquiterpene	*α*-Calacorene	0.02		1548	1548
93	40.74	Sesquiterpenoid	Elemol	0.10	0.08	1554	1554
94	41.20	Sesquiterpenoid	Nerolidol	0.11	0.02	1566	1566
95	41.93	Sesquiterpenoid	Spathulenol	0.18	0.02	1584	1584
96	42.17	Sesquiterpenoid	Caryophyllene oxide	0.09		1590	1590
97	43.17	Aldehyde	Tetradecanal	0.04		1616	1616
98	43.99	Sesquiterpenoid	*γ*-Eudesmol	0.23	0.02	1638	1637
99	44.22	Sesquiterpenoid	Ledene oxide-(II)	0.02		1644	1646
100	44.38		*Unidentified*	0.15		1649	
101	44.72	Sesquiterpenoid	*β*-Eudesmol	0.12		1658	1658
102	44.81	Sesquiterpenoid	*α*-Eudesmol	0.23	0.01	1660	1660
103	49.85	Hydrocarbon	Octadecane	0.03		1800	1800

104	50.58	Aldehyde	Hexadecanal	0.02		1821	1820
105	55.24		*Unidentified*	0.06		1961	
106	56.90	Aldehyde	Octadecanal	0.03		2013	2013
107	59.64	Hydrocarbon	Heneicosane	0.03		2101	2100

RT: Retention time RI: Retention indices calculated against LRI: Literature retention indices based on Adams, 2007, NIST and WILLEY

A total of 98 compounds, whose structures were clarified, were classified as 8 groups, for the analysis of unripe fruits of the *P. terebinthus* plant. Those detected from these classes and the number of compounds they contain were determined as aldehydes 14, hydrocarbons 5, monoterpenes 21, monoterpenoids 22, sesquiterpenes 22, sesquiterpenoids 8, others 2, and unidentified compounds 4, respectively. As a result of the analysis of the unripe fruits of terebinth plants, the most abundant chemical classes were monoterpenes (87.0%), sesquiterpenes (5.23%), and monoterpenoids (4.82%). According to the results of the analysis made on the ripe fruit samples, 71 compounds whose structures were clarified were classified as 8 groups. Those identified from these classes and the number of components they contain were aldehydes 6, hydrocarbons 3, monoterpenes 20, monoterpenoids 14, sesquiterpenes 20, sesquiterpenoids 5, and others 3. As a result of the analysis of ripe fruit samples of *P. terebinthus* plant, the most common chemical classes were monoterpenes (94.4%), sesquiterpenes (3.79%), and monoterpenoids (0.80%).

CONCLUSIONS

1. In the present work, vitamin C amounts of ripe and unripe fruits of terebinth plants were 63.2 mg/100 g and 15.4 mg/100 g, respectively. It is seen that the amount of vitamin C in the unripe fruit of the plant is higher than in the ripe fruit. The main phenolic compounds of unripe and ripe fruits are rutin, syringic acid, and gallic acid. It was determined that the enzyme inhibitor activities in the ripe and unripe fruits of terebinthus plants were 0.136 mg/mL and 2.14 mg/mL, respectively. It has been determined that unripe fruits are more effective in enzyme inhibition. A lower IC_{50} value of the sample results in more effective enzyme inhibition.
2. The antioxidant capacities of unripe-ripe samples were determined. In all of the DPPH, ABTS, FRAP, TPC, TFC, and TAC methods of *P. terebinthus* parts, it was determined that the methanol extracts attained from the ripe fruits showed higher antioxidant properties than the methanol extracts obtained from the unripe fruits.
3. Regarding the results of the antimicrobial activity analysis of *P. terebinthus* ripe fruit samples, it was determined that they demonstrated antimicrobial activity against *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 microorganisms. However, according to the results of the antimicrobial activity analyses of *P. terebinthus* unripe fruit samples, it was determined that they did not show antimicrobial activity against the microorganisms used.
4. In the GC-MS/FID analysis of the obtained essential oils, 98 and 71 compounds were detected in unripe and ripe fruits, respectively. The chemical classes with the most compounds in the essential oils of *P. terebinthus* were monoterpenoids, sesquiterpenes, and monoterpenes in unripe and ripe fruits, respectively. The chemical

classes with the highest percentage of constituents in essential oils of plant components were monoterpenes with 87.02% and 94.40% in unripe and ripe fruits, respectively. The main component found in essential oils of plant parts was α-pinene in unripe and ripe fruits with 22.82% and 27.27% ratios, respectively.

REFERENCES CITED

Abid, M., Jabbar, S., Wu, T., Hashim, M. M., Hu, B., Lei, S., Zhang, X., and Zeng, X. (2013). "Effect of ultrasound on different quality parameters of apple juice," *Ultrason Sonochemistry* 20(5), 1182-1187. DOI: 10.1016/j.ultsonch.2013.02.010

Adams, R. P. (2007). *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*, 4th Ed., Allured publishing Corporation, Carol Stream, IL, USA.

Ahmed, D., Khan, M. M., and Saeed, R. (2015). "Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from *Adiantum caudatum* leaves," *Antioxidants* 4(2), 394-409. DOI: 10.3390/antiox4020394

Akpulat, S., Tıraş, M., Şahinkaya, M. S., and Akpulat, H. A. (2021). "Antimicrobial effect of *Pistacia terebinthus* (Turpentine tree) Galls and GC-MS analysis," *Turkish Journal of Biodiversity* 4(2), 98-104. DOI: 10.38059/biodiversity.100001

Ayad, R., Amessis-Ouchemoukh, N., Guenaoui, N., Sadou, D., Madani, K., and Ouchemoukh, S. (2023). "Pomological analyses, physicochemical properties, antioxidant activities, and microbial inhibition of *Pistacia lentiscus* berries during ripening," *Erwerbs-Obstbau*, 1-13. DOI: 10.1007/s10341-023-00915-7

Baytop, T. (1999). *Treatment with Medicinal Plants in Turkey*, 2nd Edition, İstanbul: Nobel Tıp Bookstores, Istanbul, Turkey.

Bozorgi, M., Memariani, Z., Mobli, M., Surmaghi, M. H. S., Shams-Ardekani, M. R., and Rahimi, R. (2013). "Five *Pistacia* species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk* and *P. lentiscus*): A review of their traditional uses, phytochemistry, and pharmacology," *The Scientific World Journal* 2013, article ID 219815. DOI: 10.1155/2013/219815

Costa, A. G. V., Garcia-Diaz, D. F., Jimenez, P., and Silva, P. I. (2013). "Bioactive compounds and health benefits of exotic tropical red-black berries," *Journal of Functional Foods* 5(2), 539-549. DOI: 10.1016/j.jff.2013.01.029

Couladis, M., Özcan, M., Tzakou, O., and Akgül, A. (2003). "Comparative essential oil composition of various parts of the turpentine tree (*Pistacia terebinthus* L.) growing wild in Turkey," *J. Sci. Food Agriculture* 83(2), 136-138. DOI: 10.1002/jsfa.1295

Dranca, F., and Oroian, M. (2016). "Optimization of ultrasound-assisted extraction of total monomeric anthocyanin (TMA) and total phenolic content (TPC) from eggplant (*Solanum melongena* L.) peel," *Ultrason Sonochemistry* 31, 637-646. DOI: 10.1016/j.ultsonch.2015.11.008

Dubey, S., Ganeshpurkar, A., Bansal, D., and Dubey, N. (2013). "Experimental studies on bioactive potential of rutin," *Chronicles of Young Scientists* 4(2), 152-157. DOI: 10.4103/2229-5186.115556

Durak, M. Z., and Uçak, G. (2015). "Solvent optimization and characterization of fatty acid profile and antimicrobial and antioxidant activities of Turkish *Pistacia terebinthus* L. extracts," *Turkish J. Ag. For.* 39(1), 10-19. DOI: 10.3906/tar-1403-63

Durmaz, G., and Gökmen, V. (2011). "Changes in oxidative stability, antioxidant capacity and phytochemical composition of *Pistacia terebinthus* oil with roasting," *Food Chemistry* 128(2), 410-414. DOI: 10.1016/j.foodchem.2011.03.044

Farhat, M. B., Landoulsi, A., Chaouch-Hamada, R., Sotomayor, J. A., and Jordán, M. J. (2013). "Characterization and quantification of phenolic compounds and antioxidant properties of *Salvia* species growing in different habitats," *Industrial Crops and Products* 49, 904-914. DOI: 10.1016/j.indcrop.2013.06.047

Foddai, M., Kasabri, V., Afifi, F. U., Azara, E., Petretto, G. L., and Pintore, G. (2015). "*In vitro* inhibitory effects of Sardinian *Pistacia lentiscus* L. and *Pistacia terebinthus* L. on metabolic enzymes: Pancreatic lipase, α-amylase, and α-glucosidase," *Starch-Stärke* 67(1-2), 204-212. DOI: 10.1002/star.201400068

Gogus, F., Ozel, M. Z., Kocak, D., Hamilton, J. F., and Lewis, A. C. (2011). "Analysis of roasted and unroasted *Pistacia terebinthus* volatiles using direct thermal desorption GCxGC–TOF/MS," *Food Chemistry* 129(3), 1258-1264. DOI: 10.1016/j.foodchem.2011.05.003

Hacıbekiroğlu, I., Yılmaz, P. K., Haşimi, N., Kılınç, E., Tolan, V., and Kolak, U. (2015). "*In vitro* biological activities and fatty acid profiles of *Pistacia terebinthus* fruits and *Pistacia khinjuk* seeds," *Natural Product Research* 29(5), 444-446. DOI: 10.1080/14786419.2014.947492

Kasangana, P. B., Haddad, P. S., and Stevanovic, T. (2015). "Study of polyphenol content and antioxidant capacity of *Myrianthus arboreus* (Cecropiaceae) root bark extracts," *Antioxidants (Basel)* 4(2), 410-426. DOI: 10.3390/antiox4020410

Kavak, D. D., Altıok, E., Bayraktar, O., and Ülkü, S. (2010). "*Pistacia terebinthus* extract: As a potential antioxidant, antimicrobial and possible *β*-glucuronidase inhibitor," *Journal of Molecular Catalysis B: Enzymatic* 64(3-4), 167-171. DOI: 10.1016/j.molcatb.2010.01.029

Khan, H., Amin, S., Kamal, M. A. and Patel, S. (2018). "Flavonoids as acetylcholinesterase inhibitors: Current therapeutic standing and future prospects," *Biomed. Pharmacotherapy* 101, 860–870. DOI: 10.1016/j.biopha.2018.03.007

Kılıc Pekgözlü, A., and Ceylan, E. (2021). "Effect of heat treatment on the turpentine composition," *Journal of Bartin Faculty of Forestry* 23(3), 878-884. DOI: 10.24011/barofd.994641

Kim, D. H., Yang, W. T., Cho K. M., and Lee, J. H. (2020). "Comparative analysis of isoflavone aglycones using microwave-assisted acid hydrolysis from soybean organs at different growth times and screening for their digestive enzyme inhibition and antioxidant properties," *Food Chemistry* 305, article ID 125462. DOI: 10.1016/j.foodchem.2019.125462

Küçük, M., Güleç, C., Üçüncü, O., Yaşar, A., Gaydan, F., Coşkunçebi, K., Terzioğlu, S. and Yaylı, N. (2006). "Chemical composition and antimicrobial activies of the essential oils of *T. chamaedrys* subsp. *chamaedrys*, *T. orientale* var. *puberulens*, and *T. chamaedrys* subsp. *lydium*," *Pharmaceutical Biology* 44(8), 592-599.

Lima, V. N., Oliveira-Tintino, C. D., Santos, E. S., Morais, L. P., Tintino, S. R., Freitas, T. S., Geraldo, Y. S., Pereira, R. L.S., Cruz, R. P., Menezes, I. R.A., and Coutinho, H. D. M. (2016). "Antimicrobial and enhancement of the antibiotic activity by phenolic compounds: Gallic acid, caffeic acid and pyrogallol," *Microbial Pathogenesis* 99, 56-61. DOI: 10.1016/j.micpath.2016.08.004

Llorens-Molina, J. A. and Vacas, S. (2015). "Seasonal variations in essential oil of aerial parts and roots of an *Artemisia absinthium* L. population from a Spanish area with

supramediterranean climate (Teruel, Spain)," *Journal of Essential Oil Research* 27(5), 395-405. DOI: 10.1080/10412905.2015.1043400

Ma, Y.-Q., Chen, J.-C., Liu D.-H., and Ye, X.-Q. (2009). "Simultaneous extraction of phenolic compounds of citrus peel extracts: Effect of ultrasound," *Ultrason Sonochemistry* 16(1), 57-62. DOI: 10.1016/j.ultsonch.2008.04.012

Matuschek, E., Brown, D. F. J., and Kahlmeter, G. (2014). "Development of the EUCAST disk diffusion antimicrobial susceptibility testing method and its implementation in routine microbiology laboratories," *Clinical Microbiology and Infection* 20(4), 255-266. DOI: 10.1111/1469-0691.12373

Medveckienė, B., Kulaitienė, J., Levickienė, D., and Hallmann, E. (2021). "The effect of ripening stages on the accumulation of carotenoids, polyphenols and vitamin C in rosehip species/cultivars," *Applied Sciences* 11(15), 1-15, article ID 6761. DOI: 10.3390/app11156761

Mollica, A., Stefanucci, A., Zengin, G., Locatelli, M., Macedonio, G., Orlando, G., Ferrante, C., Menghini, L., Recinella, R., Leone, S., *et al.* (2018). "Polyphenolic composition, enzyme inhibitory effects *ex-vivo* and *in-vivo* studies on two Brassicaceae of north-central Italy," *Biomedicine and Pharmacotherapy* 107, 129-138. DOI: 10.1016/j.biopha.2018.07.169

Okumura, N., Yoshida, H., Nishimura, Y., Kitagishi, Y., and Matsuda, S. (2012). "Terpinolene, a component of herbal sage, downregulates AKT1 expression in K562 cells," *Oncology Letters* 3(2), 321-324. DOI: 10.3892/ol.2011.491

Orhan, I. E., Senol, F. S., Gulpinar, A. R., Sekeroglu, N., Kartal M., and Sener, B. (2012). "Neuroprotective potential of some terebinth coffee brands and the unprocessed fruits of *Pistacia terebinthus* L. and their fatty and essential oil analyses," *Food Chemistry* 130(4), 882-888. DOI: 10.1016/j.foodchem.2011.07.119

Öz, M., Baltacı, C., and Deniz, İ. (2018). "The analysis of sugar and vitamin C in rosehip (*Rosa canina* L.) and black rosehip (*Rosa pimpinellifolia* L.) fruits of Gumushane region," *Gümüşhane University Journal of Science and Technology* 8(2), 284-292. DOI: 10.17714/gumusfenbil.327635

Özcan, M. M., Al Juhaimi, F., Uslu, N., Ahmed, I. A. M., Babiker, E. E., Osman, M. A., Gassem, M. A., Alqah, H. A. S., and Ghafoor, K. (2020). "Effect of sonication process of terebinth (*Pistacia terebinthus* L.) fruits on antioxidant activity, phenolic compounds, fatty acids and tocopherol contents," *Journal of Food Science and Technology* 57(6), 2017-2025. DOI: 10.1007/s13197-019-04235-1

Özcan, M. M., Tzakou, O., and Couladis, M. (2009). "Essential oil composition of the turpentine tree (*Pistacia terebinthus* L.) fruits growing wild in Turkey," *Food Chemistry* 114(1), 282-285. DOI: 10.1016/j.foodchem.2008.08.094

Öztürk, R. (2008). "Nosocomial infections: Problems, new targets and legal responsibility," in: *Hospital Infections Prevention and Control Symposium* (Symposium Book), Publications of İstanbul University Faculty of Medicine, Istanbul, Turkey, pp. 23-29.

Paje, L. A., Choi, J., Lee, H. D., Kim, J., Yu, A. R., Bae, M. J. and Lee, S. (2022). "Phenolic acids and flavonoids from *Salvia plebeia* and HPLC-UV profiling of four *Salvia* species," *Heliyon* 8(3), article ID e09046. DOI: 10.1016/j.heliyon.2022.e09046

Puvača, N., Čabarkapa, I., Petrović, A., Bursić, V., Prodanović, R., Soleša, D., and Lević, J. (2019). "Tea tree (*Melaleuca alternifolia*) and its essential oil: Antimicrobial, antioxidant and acaricidal effects in poultry production," *World's Poultry Science Journal* 75(2), 235-246. DOI: 10.1017/S0043933919000229

Rauf, A., Patel, S., Uddin, G., Siddiqui, B. S., Ahmad, B., Muhammad, N., Mabkhot, Y. N. and Hadda, T. B. (2017). "Phytochemical, ethnomedicinal uses and pharmacological profile of genus *Pistacia*," *Biomedicine and Pharmacotherapy* 86, 393-404. DOI: 10.1016/j.biopha.2016.12.017

Sağdıç, O., Ozturk, I., Ozkan, G., Yetim, H., Ekici, L., and Yilmaz, M. (2011). "RP-HPLC-DAD analysis of phenolic compounds in pomace extracts from five grape cultivars: Evaluation of their antioxidant, antiradical and antifungal activities in orange and apple juices," *Food Chemistry* 126(4), 1749-1758.

Stanojević, L. P., Todorović, Z. B., Stanojević, K. S., Stanojević, J. S., Troter, D. Z., Nikolić, L. B., and Đorđević, B. (2021). "The influence of natural deep eutectic solvent glyceline on the yield, chemical composition and antioxidative activity of essential oil from rosemary (*Rosmarinus officinalis* L.) leaves," *Journal of Essential Oil Research* 33(3), 247-255. DOI: 10.1080/10412905.2021.1873867

Topçu, G., Ay, M., Bilici, A., Sarıkürkçü, C., Öztürk, M., and Ulubelen, A. (2007). "A new flavone from antioxidant extracts of *Pistacia terebinthus*," *Food Chemistry* 103(3), 816-822. DOI: 10.1016/j.foodchem.2006.09.028

Usai, M., Pintore, G., Chessa, M., and Tirillini, B. (2006). "Essential oil composition of different aerial parts of *Pistacia terebinthus* L. growing wild in Sardinia," *Journal of Essential Oil Research* 18(4), 383-385. DOI: 10.1080/10412905.2006.9699121

Uyar, A., and Abdulrahman, N. T. (2020). "A histopathological, immunohistochemical and biochemical investigation of the antidiabetic effects of the *Pistacia terebinthus* in diabetic rats," *Biotechnic and Histochemistry* 95(2), 92-104. DOI: 10.1080/10520295.2019.1612092

Uysal, S., Sinan, K. I., Jekő, J., Cziáky, Z., and Zengin, G. (2022). "Chemical characterization, comprehensive antioxidant capacity, and enzyme inhibitory potential of leaves from *Pistacia terebinthus* L. (Anacardiaceae)," *Food Bioscience* 48(1-7), article ID 101820. DOI: 10.1016/j.fbio.2022.101820

Yang, D., Wang, L., Zhai, J., Han, N., Liu, Z., Li, S., and Yin, J. (2021). "Characterization of antioxidant, α-glucosidase and tyrosinase inhibitors from the rhizomes of *Potentilla anserine* L. and their structure activity relationship," *Food Chemistry* 336, article ID 127714. DOI: 10.1016/j.foodchem.2020.127714

Yu, Z., Yin, Y., Zhao, W., Liu, J., and Chen, F. (2012). "Anti-diabetic activity peptides from albumin against α-glucosidase and α-amylase," *Food Chemistry* 135(3), 2078-2085. DOI: 10.1016/j.foodchem.2012.06.088

Zahrani, N. A. A., El-Shishtawy, R. M., and Asiri, A. M. (2020). "Recent developments of gallic acid derivatives and their hybrids in medicinal chemistry: A review," *European Journal of Medicinal Chemistry* 204, 1-37, article ID 112609. DOI: 10.1016/j.ejmech.2020.112609

Zengin, G., Rodrigues, M. J., Abdallah, H. H., Custodio, L., Stefanucci, A., Aumeeruddy, M. Z., Mollica, A., Rengasamy, K. R. R., and Mahomoodally, M. F. (2018). "Combination of phenolic profiles, pharmacological properties and *in silico* studies to provide new insights on *Silene salsuginea* from Turkey," *Computational Biology and Chemistry* 77, 178-186. DOI: 10.1016/j.compbiolchem.2018.10 .005

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